Seed and Root Exudations in Relation to *Rhizoctonia* Damping-off of Various Crops[1]

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Introduction

The relation between plant exudates and soil-borne diseases is a challenging problem in the field of plant pathology. Barton (1) and other workers (2,3,4,5,67,8,9,10,13,) indicated that exudates from roots or other plant parts are attractive to parasitic organisms, or inductive to a growth response of pathogens that aids the infection process. Schroth and Snyder (11) proved that amino acids and sugars of bean exudates favorably influenced germination and growth of chlamydospores of *Fusarium solani* f. *phaseoli* in soil. Schroth and Cook (14) reported that plant exudate was not specific to *Fusarium* spp. since spores of may other fungi germinated contiguously to seed germination of bean, and they evidently proved that incidence of pre—emergence damping—off of bean caused by *Rhizoctonia* sp. (isolates of the *Praticola* type) and *Pythium* spp. was greatly influenced by the amount of seed and young root exudation. Schroth and Hendrix (12) also found that chlamydospores of *F. solani* f. *phaseoli* germinated in the presence of exudates from many nonsusceptible plants as well as the host plant. On the other hand, the specificity of root exudates in stimulating plant pathogen was demonstrated by Kerr (8). He grew sterile seedlings in cellophane bags and buried in the soil inoculated with *Pellicularia filamentosa* (=*Rhizoctonia solani*). An intense development of the pathogen occurred on the cellophane opposite to the roots of the two susceptible hosts of lettus and radish, but such effect was not observed with tomato roots, which are not susceptible to the pathogen. Buxton (2) also reported a specific relationship between the germination of spores of *F. oxysporum* f. *pisi* and the exudates of three pea varieties differing in susceptibility to this pathogen. Although further understanding of the dynamic situation operating in the rhizosphere is needed, it is almost certain that root exudates of plants play an important role in the establishment and maintaince of the rhizosphere microorganism populations.

The experiments reported herein were made to determine whether the amount of seed exudations would vary among different susceptible host plants and whether the exudates would influence growth of *R. solani* mycelium and incidence of pre—and post—

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emergence damping- off. Consideration was also given to the sites of seed and young root exudations.

Materials and Methods

Nineteen crops varying in susceptibility to *Rhizoctonia solani* were used in this study. The isolate of *R. solani* was obtained in 1968 from kenaf seedlings. The fungus was maintained through serial transfers on potato—sucrose agar (PSA) . Each hyphal cell of the isolate was found to be multinucleate.

The susceptibility of different plants to *R. solani* was studied by planting seeds in autoclaved soil infested with mycelial suspension. The soil was contained in greenhouse flats. Four hundred seeds of each crop were planted in 20 rows (20 seeds/row) in a completely randomized design. The number of seeds germinated was recorded 10 days after planting.

The filter paper technique (11) was applied for chemical detection of substances exuded from seeds and seedlings. A piece of 7×10—in. Whatman No. 3MM filter paper was inserted between two pieces of same size of aluminum foil and autoclaved for 15 minutes at 250°C. Seeds of different crops were first treated in a solution of 10% Clorox (sodium hypochlorite) and 2% ethanol for 4 minutes, then placed on the filter paper 1½ in. apart the edge. The paper was moistened with sterile distilled water and placed in a sterilized 1×10×8—in. plastic container. Sterilized nonabsorbant cotton was used to press the foil snugly against the seeds and the young seedlings, to encourage roots to grow flush with the paper. The container was placed in a polythylene bag, kept in the dark, and canted at 60° angle to induce the roots to grow downward and against the paper. Materials exuded from plant parts were absorbed by the filter paper. After 5 days, the germinated seeds were removed and tested for contamination by placing them in Petri dishes containing PSA. The filter paper was dipped in ninhydrin color reagent to detect the exudation of amino acids, amides, and amines. Silver nitrate was used to detect the exudation of reducing sugars. Fifty replications were made for each crop.

Root exudates from the seedlings of various crops were collected to determine their effects on the mycelial growth of *R. solani*. Colorox-treated seeds were planted in sterile 500—ml flasks (5 per flask) containing purified sand that had been acid washed and ignited. After 10 days on the laboratory bench, seedlings were carefully removed and each flask was tested for contamination by placing segments of seedlings and a small amount of sand in Petri dishes containing PSA. The contents of 10 flasks were washed several times with sterile water. The washings were filtered to eliminate root retritus and evaporated to 10 ml. The eluate was then centrifuged in a Spinco refrigerated ultracentrifuge at 35,000 rpm for 30 minutes. This procedure gave a clear supernatant liquid virtually free of bacteria and fungi. Twenty ml of the concentrate were poured into a sterile 50 —ml flask and inoculated with 1 ml mycelial suspension of *R. solani*. After 5 days of incubation at 28°C, the colony was filtered for measurement of mycelial dry weight. Solid media were prepared by adding 2% of agar powder into the concentrate. Each Petri dish containing 20 ml of the medium was inoculated with a 3 mm—diam. of mycelial

disc. The colony diameters were measured after 2 days of incubation at 28°C.

For determining the effect of exudates on the hyphal growth of *R. solani* in soil after planting various crops, autoclaved sandy loam contained in plastic pots was either infested with 5 ml mycelial suspension per pot or remained uninfested to serve as control. After planting of seeds, 4 pieces of slides coated with water agar were inserted into a pot at different directions. All pots were kept in the laboratory where the temperature fluctuated around 28±2°C and provided with 10 hr. illumination. Five replicates were made for each crop and the test was repeated three times.

The water—agar—coated slides were carefully removed from pots 7 days after inoculation and passed through heat fixing, running water washing, and finally stained with Rose bengal—phenol solution. Percentage of hyphal appearance on each slide was counted by examining 350 microscopic fields under a low power (×100) microscope.

Results

Susceptibility of 19 different crops to *Rhizoctonia solani* :

Of the 19 different kinds of crop (Table 1) tested, no pre—emergence damping—off (including seed rot) was observed with cabbage, sorghum, corn, radish, broccoli, rice, and cotton. Comparatively severe incidence of the disease was found on peanut, carrot, soybean, mungbean, jute, and pea, and the percentages of diseased plants were 78.9%, 50.0%, 33.3%, 25.7%, and 22.0%, respectively. The other crops, including tomato, onion, broadbean, garlic, wheat, and kenaf, showed less than 13.0% infection.

Seedlings of peanut, garlic, broadbean, rice, and wheat were free from the attack of *R. solani*. However, seedlings of mungbean, soybean, and cotton were completely destroyed within 24 days following seed germination. The percentages of seedlings of the other crops killed by the fungus ranged from 2.5% to 75.0%. Detailed results are shown in Table 1.

Comparison of regions of exudation in seedlings of 19 different plants :

Development of the filter paper with ninhydrin showed that exudation was primarily from germinating seeds and/or root tips of primary, lateral and adventious roots depending upon the kinds of plants. The exudation of ninhydrin—reacting materials in the plants tested appeared to be particularly heavy from seeds (Fig. 3) . The source of the exudates was found to be in the vicinity of the apical meristem, region of the elongation, and region of developing root hairs of the seedlings of broadbean, radish, sorghum, corn, kenaf, soybean, tomato, broccoli, and especially mungbean. Amounts of ninhydrin—reacting materials varied from crop to crop. According to the reacted areas on the filter paper, the greatest amount of ninhydrin—reacting materials was exudated from seeds and seedlings of peanut and broadbean, while only trace amount of the exudates was obtained with rice, cabbage, mungbean, radish, carrot, and tomato (Fig. 1) .

Table 1. Percentages of pre—and post—emergence damping—off of various crops grown on the soil infested with *Rhizoctonia solani*@

Crop	No. of seeds planted	Corrected germination percentage (%)	Pre—emergence damping—off (%)	Post—emergence damping—off (%)
Cabbage	400	100.0	0	10.0
Sorghum	400	100.0	0	73.7
Pea	400	78.0	22.0	17.4
Jute	400	74.3	25.7	60.0
Mungbean	400	74.3	25.7	100.0
Corn	400	100.0	0	2.5
Radish	400	100.0	0	40.0
Onion	400	93.6	6.5	75.9
Carrot	400	50.0	50.0	14.3
Peanut	400	21.1	79.0	0
Garlic	400	95.0	5.0	0
Broadbean	400	94.1	5.9	0
Broccoil	400	100.0	0	14.3
Rice	400	100.0	0	0
Wheat	400	97.1	2.9	0
Tomato	400	87.5	12.5	14.3
Soybean	400	66.7	33.3	100.0
Kenaf	400	97.1	2.9	11.8
Cotton	400	100.0	0	100.0
LSD 5%			0.04	0.08
1%			0.07	0.14

@Including seed rot. Total percentage of damping-off seedlings in 24 days following germination.

A similar pattern of exudation was obtained by developing the filter paper with color reagents specific for sugars. Exudation of silver nitrate—reacting materials was also highly variable among crops (Fig. 1 and 4) . The major sites of exudation were from germinating seeds and young roots. However, sugar exudation of mungbean was different from the other crops, and was mainly from roots. It was obvious that broadbean, garlic, and wheat exudated greater amounts of sugars than the other crops. No sugar exudation was detected from rice, onion, and carrot.

Influence of root exudates on mycelial growth of *Rhizoctonia solani*

The mycelial density of *Rhizoctonia solani* in the soil changed considerably after planting of plants (Fig. 2) . The initial percentage of hyphal appearance of *R. solani* in the soil was 2.4%, then increased to 12.1%, 11.3%, and 8.0%, respectively, 10 days after

planting pea, mungbean, and soybean. Seedling of cotton, rice, sorghum, broadbean, peanut, tomato, and onion also increased the mycelial density to some extents. However, some crops such as garlic, jute, cabbage, radish, broccoli, corn, and especially wheat reduced the mycelial density of the organism in the soil.

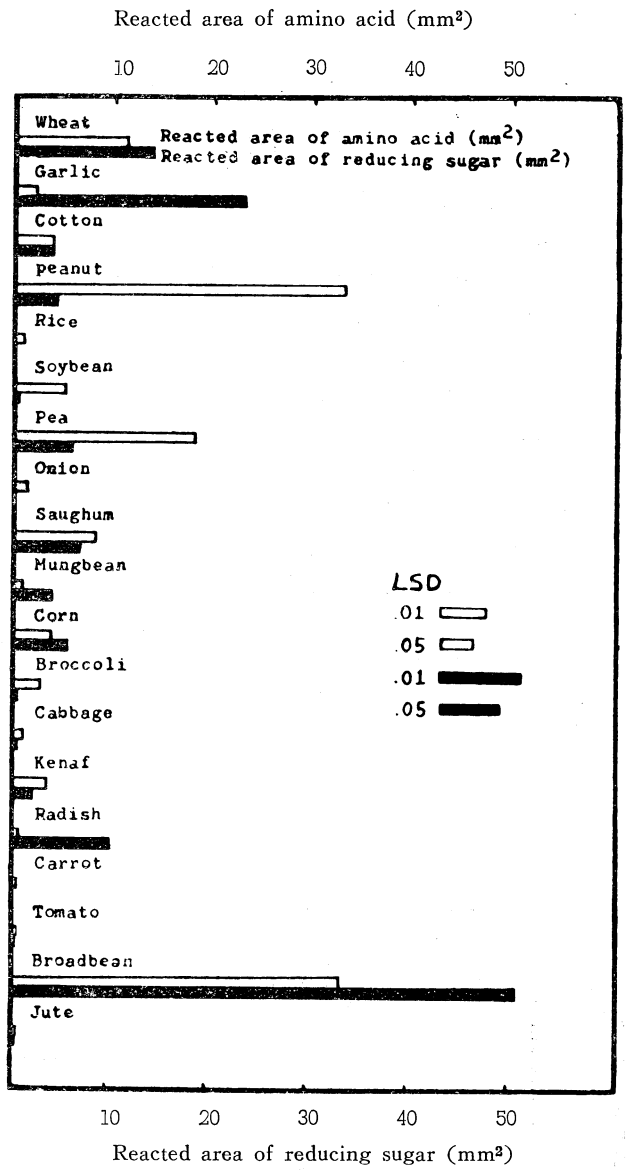
Table 2. Growth of *Rhizoctonia solani* on various root—exudates at 28°C.

Crop Root—exudate	Avg. colony diameter (mm) after 2 days of growth	Avg. mycelial dry weight (mg) after 5 days of growth
Cabbage	64.15	0.07
Pea	61.25	0.27
Jute	63.75	0.42
Corn	62.25	0.37
Radish	68.62	0.22
Onion	57.12	0.22
Carrot	60.25	0.12
Broccoli	58.83	0.42
Wheat	58.25	0.72
Tomato	64.00	0.12
Soybean	64.25	0.09
Kenaf	65.25	0.39
Cotton	68.00	0.37
CK	60.37@	0.37@@
LSD 5%	6.32	0.04
1%	8.47	0.06

@2% distilled water agar.
@@Distilled water.

Similar results were obtained from the cultivation of *R. solani* with root exudates of various crops (Table 2) . *R. solani* grew well on the medium containing radish and cotton root exudates. The colony diameter of *R. solani* on these two root—exudate media measured 68.62 and 68.00 mm, and that on water—agar medium only reached 60.37 mm. The radial growth of *R. solani* seemed to be inhibited on the media containing broccoli—, wheat—, or onion—root exudate. Although the radial growth of the organism on wheat—root exudate was poor, it was rather compact and the dry weight of the colony was higher than those on other root—exudate media.

Figure 1. Chemical detection of substances exudated from seeds and seedlings of various crops at 5 days after germination



Discussion

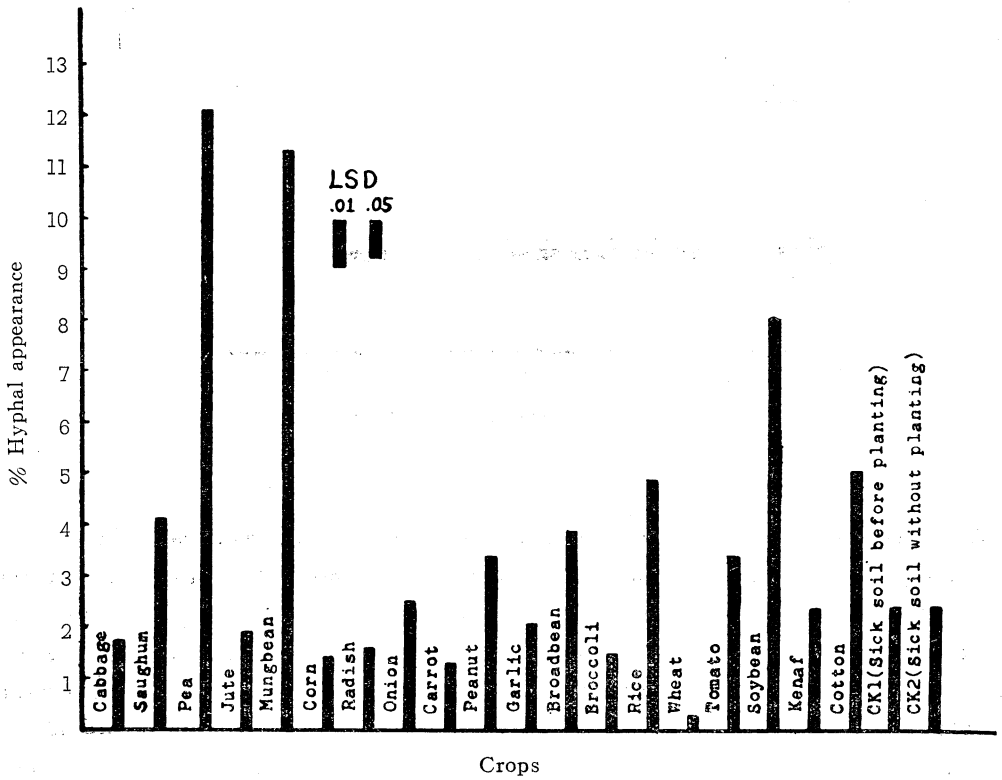
This study indicates that the susceptibility of plant to *Rhizoctonia solani* can be classified into four groups : a) both seeds and seedlings are susceptible, this group includes soybean, jute, mungbean,onion, carrot, tomato, pea, and kenaf ; b) seeds are susceptible, they are garlic, broadbean, and wheat ; c) seedling are susceptible, they are cabbage, sorghum, corn, radish, broccoli, and cotton ; and d) both seeds and seedlings are resistant, this group includes rice only. It was also found that the susceptibility of seeds of different crops was in the order of peanut, carrot, soybean, mungbean, jute, pea, tomato, onion,

broadbean, garlic, wheat, kenaf. The degree of seedling susceptibility to the disease was in the order of mungbean,soybean, cotton, onion, sorghum, jute, pea, tomato, carrot, broccoli, kenaf, cabbage.

Incidence of both pre—and post—emergence damping—off was affected by the hyphal density of *R. solani* in the soil (Table 1 and Figure 1) . There was a significant difference in increase of hyhphal density among the crops planted (Fig. 2) , and those plants stimulating hyphal density in the soil also induced hyphal growth *in vitro* with their root exudates. However, the disease incidence tended to become higher when the radial growth of colony was stimulated by the root exudates of the plants. Increase in mycelial weight appeared to have no important role on severity of the disease. The results reported herein agree with Kerr's observation (8) that radish is one of mycelial growth stimulators of *R. solani in vitro* (Table2) , but this is not always found to be true *in vivo* (Table 1 and Figure 2) . It is presumed that the ability of stimulation is influenced by soil structure, soil physical and chemical conditions, and soil microflora rather than plant root exudates alone.

Schroth and Cook (14) reported that the quantity of seed exudation was an important factor affecting the incidence of bean damping—off among varieties. In this study, however, the amount of root exudates seemed to be not so important as seed exudations.

Figure 2. Changes of hyphal density of *Rhizoctonia solani* in soil at 10 days after planting of various crops



Young roots of broadbean secreted a greater amount of both amino acids and reducing sugars among 18 different crops (Fig. 2) , but only 5.9% of the seeds were infected with the pre—emergence damping—off and no diseased seedlings were observed (Table 1) . This result indicates that the incidence of *R. solani*—damping—off is mainly affected by the nature of substances exuded. Therefore, further studies on the relationship between susceptibility of plants to *R. solani* and the nature of root exudates are necessitated.

Figure 3. Exudation patterns of ninhydrin-positive substance of various crops on filter papers

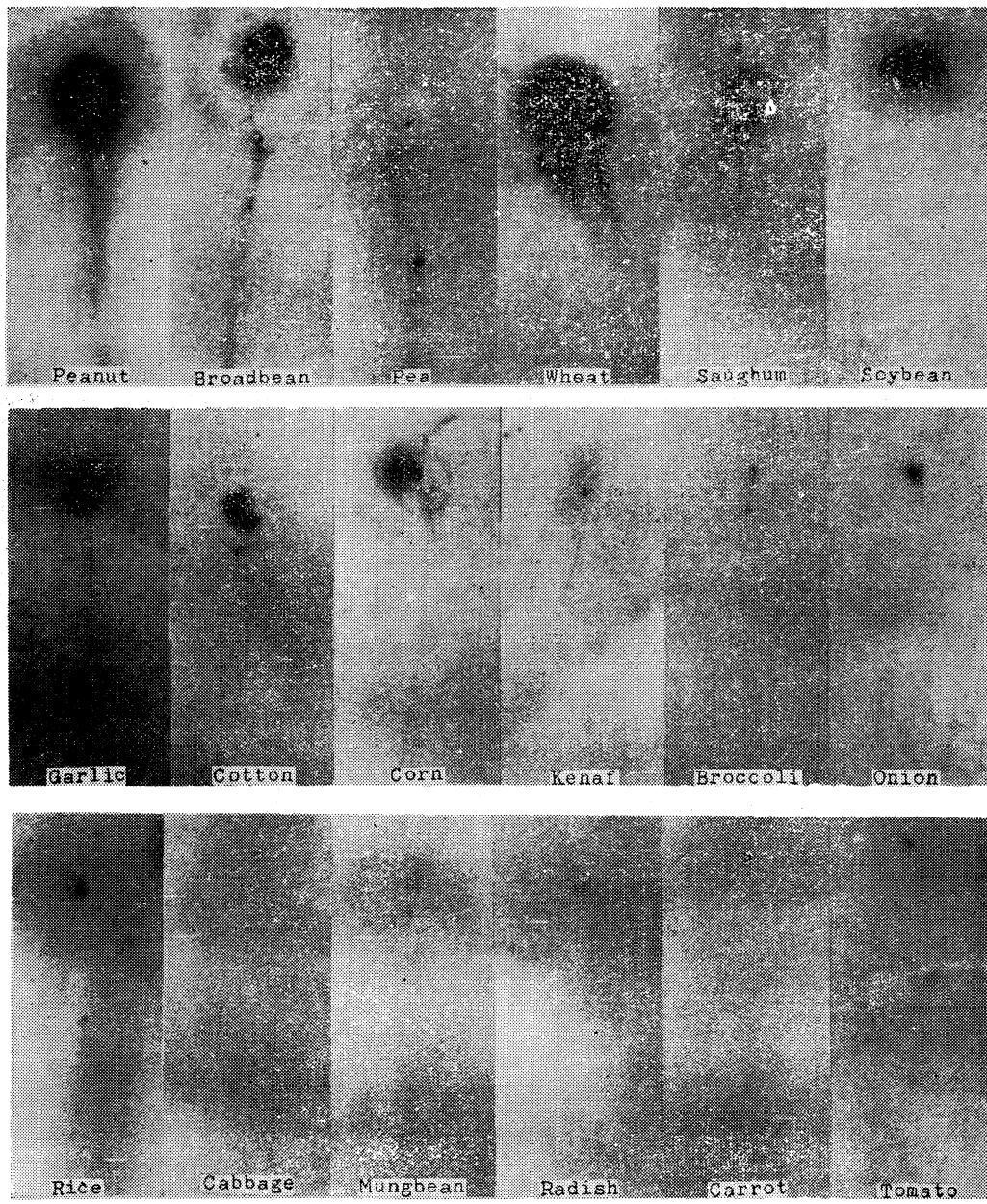
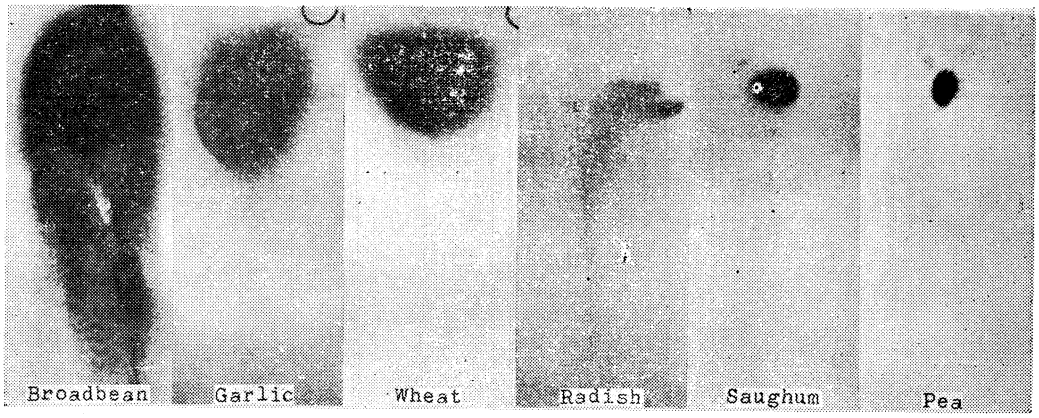
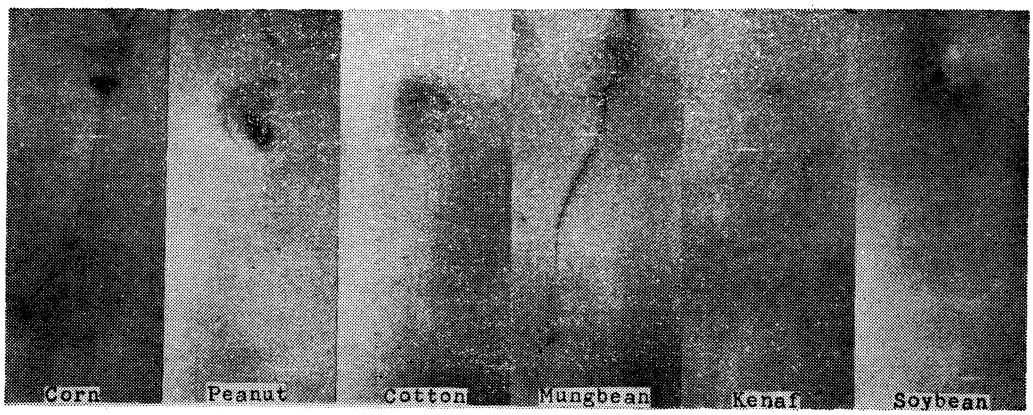
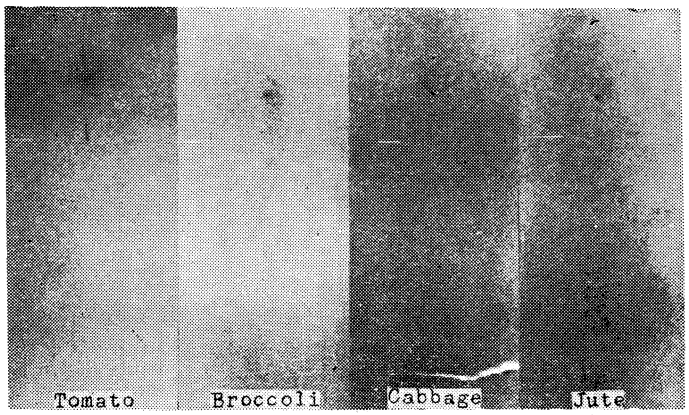


Figure 4. Exudation patterns of silver nitrate-positive substances of vrious crops on filter papers







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種子和根分泌物與某些作物發生 *Rhizoctonia* 腰折病之關係[1]

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中文摘要

植物根或其他部位之分泌物已被確認可引誘寄生性生物之發生，促進植物病原菌之生長，並有助於侵染之完成。本研究以19種對 *Rhizoctonia solani* 不同感病程度之作物，以濾紙法測定其種子和根分泌物中含有氨基酸類及低醣類之多寡和分泌部位，比較各種作物種子和根分泌物培養病原菌後對菌絲生長之影響，並與栽植各種作物後土壤內菌絲密度之實際增減情形對照觀察，以資進一步瞭解種子和根分泌物對各種作物發生 *Rhizoctonia* 腰折病之關係。

供試19種作物對 *R. solani* 菌株 K-1之 感病情形可分成四類：① 種子和幼苗均可被侵染者有大豆、黃麻、綠豆、洋葱、胡蘿蔔、番茄、豌豆、和蘇麻；②僅種子可被侵染者有大蒜、蠶豆、和小麥；③僅幼苗可被侵染者有白菜、高粱、玉米、蘿蔔、花椰菜和棉花；④種子和幼苗均有抗性者僅水稻。

各種作物氨基酸類之分泌大都源自發芽種子，主根和支根根尖、根毛、或生育中鬚支根等，因作物不同，分泌部位亦異。依據濾紙法測量各種作物在濾紙上分泌氨基酸類面積大小結果，以花生及蠶豆分泌量最多，而水稻、白菜、綠豆、蘿蔔、胡蘿蔔、和番茄則僅微量。低醣類之分泌大都源自發芽種子和幼根，惟綠豆分泌部位集中在幼根。在供試作物中低醣類分泌量以蠶豆、大蒜和小麥等 3 種作物顯著地比其他作物爲多。

作物之萌前腰折病和萌後腰折病之發生與土壤內病原菌菌絲密度有密切關係。種植後可增進 *R. solani* 在土壤內菌絲密度之作物，在室內研究中證明其根分泌物確可刺激菌絲體之生長。根分泌物分兩種情形刺激菌絲體生長，促進菌絲平面生長速度或增進菌絲體乾重量。菌絲體乾重量之增加對罹病程度之加劇和土壤內菌絲密度之增大，並無直接而明顯之關連。

本研究之完成得國家長期科學發展委員會之補助。

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2 臺灣省農業試驗所臺南棉麻試驗分所技正兼植物保護系主任